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Δ^9-Tetrahydrocannabinol inhibits gastric motility in the rat through cannabinoid CB_1 receptors

Zbigniew K. Krowicki *, Joseph M. Moerschbaecher, Peter J. Winsauer, Sivarao V. Digavalli, Pamela J. Hornby

Department of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112, USA

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Abstract

We investigated involvement of the autonomic nervous system in gastric motor and cardiovascular responses to Δ^9-tetrahydrocannabinol (Δ^9-THC) in anesthetized rats. Intravenously administered Δ^9-THC evoked long-lasting decreases in intragastric pressure and pyloric contractility, bradycardia, and hypotension. The changes in gastric motor function and bradycardia were abolished by vagotomy and ganglionic blockade, whereas spinal cord transection prevented the hypotensive response. Administered intravenously alone, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, a putative cannabinoid CB_1 receptor antagonist, evoked transient decrease in intragastric pressure, and hypertension that was associated with bradycardia. However, this agent completely blocked the gastric motor and cardiovascular responses to intravenous Δ^9-THC. Application of Δ^9-THC to the dorsal surface of the medulla resulted in small and short-lasting decreases in gastric motor and cardiovascular function. We conclude that the decrease in gastric motor function and bradycardia are partially due to an action of Δ^9-THC in the dorsal medulla and that intact vagal nerves are required. The hypotension was mediated through sympathetic pathways. Both gastric motor and cardiovascular effects of peripherally administered Δ^9-THC seem to be mediated through cannabinoid CB_1 receptors.

Keywords: Δ^9-Tetrahydrocannabinol; Blood pressure; Brainstem; Cannabidiol; Gastric motility; Heart rate; Intragastric pressure; *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide

1. Introduction

Δ^9-Tetrahydrocannabinol (Δ^9-THC), the most active psychotropic ingredient of marijuana, inhibits gastric motor function (Dewey et al., 1970; Shook et al., 1986; Shook and Burks, 1989; Varga et al., 1995), as well as gastric acid secretion (Rivas and Garcia, 1980) and gastric ulcer formation (De Souza et al., 1978) in rats. Intravenous administration of (Δ^9-THC) in the same species effectively reduced gastric emptying and small intestinal transit more than large intestinal transit, suggesting that this compound affects upper gastrointestinal tract preferentially (Shook and Burks, 1989). Since changes in gastric motility and tone are related to nausea and vomiting, and Δ^9-THC is being used as an anti-emetic agent (Mattes et al., 1993; Gonzalez-Rosales and Walsh, 1997), it is important to assess the extent to which Δ^9-THC can alter gastric motor function. Two distinct cannabinoid receptors have been cloned. The CB_1 receptor, which is predominantly located in the central nervous system (Matsuda et al., 1990), and the CB_2 receptor, located on immune cells and on peripheral tissues (Munro et al., 1993). The existence of subtypes of the cannabinoid CB_1 receptor has been recently suggested (Welch et al., 1998). In addition, the development of selective receptor agonists and antagonists have renewed interest in the sites and mechanisms of action of Δ^9-THC (Felder and Glass, 1998). This is especially relevant because both receptor subtypes bind Δ^9-THC and anandamide, which is an endogenous cannabimimetic eicosanoid (Axelrod and Felder, 1998). Specifically, with regard to their gastrointestinal effects, it has recently been reported that intestinal transit in mice is inhibited by both Δ^9-THC and anandamide, due to an action on cannabinoid CB_1 receptors (Calignano et al., 1997; Colombo et al., 1998). Therefore, in the present study, we have addressed

* Corresponding author. Tel.: +1-504-568-5176; Fax: +1-504-568-2361; E-mail: zkrowi@lsumc.edu

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the issue of whether inhibition of gastric motor function is due to cannabinoid CB_1 receptor activation by Δ^9-THC.

We were also interested in the extent to which the gastric motor inhibitory effects of Δ^9-THC were mediated via a central or peripheral site of action. This is because the cannabinoid CB_1 receptor, as well as its isoform CB_{1A} are located within both the central nervous system (Rinaldi-Carmona et al., 1996a; Tsou et al., 1998), and within the enteric nervous system of the gastrointestinal tract (Shire et al., 1995; Pertwee et al., 1996). In contrast, the cannabinoid CB_2 receptor is expressed primarily in immune tissue (Rinaldi-Carmona et al., 1996a). Within the myenteric plexus of the gut, cannabinoid CB_1 receptor agonists inhibit electrically-evoked contractions of the guinea-pig small intestine, probably by presynaptic inhibition of acetylcholine release (Pertwee et al., 1996; Lopez-Redondo et al., 1997). This suggests that the myenteric plexus is a major site of action for the gastrointestinal motor inhibitory effects of these agents in vivo. However, within the central nervous system of both rats (Mailleux and Vanderhaegen, 1992; Matsuda et al., 1993; Tsou et al., 1998) and humans (Glass et al., 1997), cannabinoid receptor mRNA is located in the dorsal vagal complex. This complex is comprised of the dorsal motor nucleus of the vagus, which is the lower brainstem site of origin of gastrointestinal parasympathetic preganglionic neurons (reviewed in Krowicki and Hornby, 1995), and the nucleus of the solitary tract (where cardiovascular baroreceptor and gastrointestinal afferents terminate) (Miselis et al., 1991; Agarwal and Calaresu, 1992). The presence of cannabinoid CB_1 receptors in the dorsal vagal complex, as well as within the myenteric plexus means that Δ^9-THC could mediate its gastrointestinal effects by a direct action in the brain, in addition to its peripheral effects. A direct action of Δ^9-THC in the dorsal vagal complex is supported by the fact that it alters the spontaneous firing rate of neurons in the nucleus of the solitary tract (Himmi et al., 1996). Therefore, these observations prompted us to investigate (1) the extent to which peripherally administered Δ^9-THC mediates gastric inhibitory effects via intact vagal and sympathetic pathways and (2) whether application of Δ^9-THC onto the dorsal surface of the medulla (immediately above the dorsal vagal complex) could evoke gastric motor effects. In addition, we also measured the cardiovascular effects of Δ^9-THC during these manipulations to compare our data with data from other studies suggesting that cannabinoid CB_1 receptors activate a sympathetic nervous system site that produces hypotension and bradycardia (Varga et al., 1995; Lake et al., 1997).

2. Materials and methods

2.1. Animals

Forty male Sprague–Dawley rats (245–395 g; Charles River Laboratories, Wilmington, MA) were used in this study, which was approved by the Louisiana State University Medical Center Institutional Animal Care and Use Committee. Food was withheld 12 h before experiments but the animals had free access to tap water.

2.2. Surgery

The animals were initially anesthetized with a mixture of ketamine and xylazine (i.m., 50 and 5 mg/kg, respectively) and separate cannulae were placed in the left femoral artery (for blood pressure recording) and vein, then α-chloralose (i.v., 80 mg/kg) was administered 25 min later. If needed, urethane (i.v., 600 mg/kg) or xylazine (i.v., 2.5 mg/kg) were used to maintain full surgical anesthesia in the presence of α-chloralose. The trachea was cannulated with PE-160 tubing to maintain an open airway. Intragastric pressure was continuously recorded using a latex balloon placed in the stomach. Additionally, two small strain gauges were mounted on the surface of the stomach for monitoring of circular smooth muscle contractile activity of the pyloric region and longitudinal smooth muscle of the greater curvature of the stomach. The catheter in the left femoral artery was connected to a pressure transducer (Viggo-Spectramed, model P23XL, Oxnard, CA) and polygraph (model 7E, Grass Instrument, Quincy, MA) for direct measurement of blood pressure. Heart rate was monitored by a tachograph driven by the pressure wave (model 7P4H, Grass Instrument).

Bilateral vagotomy was performed in six animals at the midcervical level. Briefly, the vagi were carefully separated from the left and right common carotid arteries and silk snares were loosely placed around them, then vagotomy was achieved by avulsion. The intragastric pressure is routinely readjusted after vagotomy by inflating the balloon with additional 1–2 ml of water to maintain it at levels comparable to pretreatment. In addition, we have previously demonstrated that we are able to evoke additional gastric relaxation after cholinergic blockade with atropine, which itself decreases intragastric pressure (Krowicki and Hornby, 1996).

Transection of the cervical spinal cord was performed in four animals at the level of the medullospinal transition region. To prevent activation of nociceptive reflexes, 0.5 ml of 2% lidocaine HCl (Butler, Columbus, OH) was injected with a 25-gauge needle directly into the exposed spinal cord in several locations, then 0.5 cm of the cord was excised to ensure complete interruption of spinal efferents. These acute, terminal procedures were performed in the presence of full surgical anesthesia. As a rule, vagotomy or spinal cord transection was performed after typical responses to Δ^9-THC were obtained, followed by repeat administration of the cannabinoid 30–60 min later.

Rectal temperature was maintained between 37.0 and 37.5°C with radiant heat lamp.

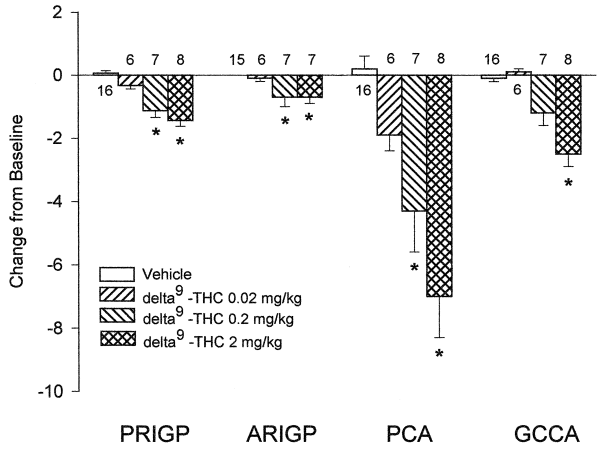

Fig. 1. Acute effects of vehicle or Δ^9-THC (0.02–2 mg/kg) administered as a bolus intravenous injection on peak intragastric pressure (PRIGP) as well as pyloric circular muscle (PCA) and greater curvature longitudinal muscle (GCCA) contractile activity. PCA and GCCA values reflect changes which occurred within 2 min after injections. Data are means ± S.E. for the number of animals indicated below or above each bar. * Statistically significant when compared with the effect of vehicle.

2.3. Treatment

Intravenous injections were delivered through the catheter in the femoral vein in a volume of 1 ml/kg. For applications to the dorsal surface of the medulla, animals were placed prone in a stereotaxic frame with the tooth bar set at −6.5 mm below the interaural line. Then, the atlantooccipital membrane was cut horizontally and adjacent occipital bones were carefully removed to expose the dorsomedial medulla. Under visual control through a stereoscopic eyeglass magnifier (Stereomax, Wetzlar, Germany), 5 μl aliquots of vehicle (see below) or Δ^9-THC solution were directly applied to the surface of the fourth ventricle, using a 10-μl Hamilton microsyringe. In some animals, a small piece of filtration paper was soaked with the same volume of vehicle or Δ^9-THC solution and placed on the dorsal surface of the medulla.

2.4. Drugs

Δ^9-THC was obtained from the National Institute on Drug Abuse. *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716A) was obtained from Sanofi (Paris, France). The range of doses tested i.v. (0.02–2.0 mg/kg, Δ^9-THC) are based on the ED_{50} values obtained in rats for producing decreases in gastric emptying (Shook and Burks, 1989) as well as bradycardia and hypotension (Lake et al., 1997). All the compounds were dissolved in 1:1:18 (emulphor:ethanol:saline). Emulphor (Alkamuls EL-620L), a polyoxyethylated vegetable oil, was purchased from the Rhone-Poulenc (Princeton, NJ). The dose of *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide and the route of administration were chosen from available literature (Compton et al., 1996; Terranova et al., 1996). Hexamethonium bromide (Sigma, St. Louis, MO) was dissolved in saline at a final concentration of 15 mg/(kg ml)$^{-1}$.

2.5. Data analysis

For peak response in intragastric pressure (maximum difference from baseline), pyloric and greater curvature minute motility indexes, heart rate, and blood pressure, the changes from baseline were calculated. Minute motility index was calculated for 2 min before and after microinjection according to Ormsbee and Bass (1976), as reported previously (Krowicki et al., 1997). Blood pressure is expressed as mean arterial blood pressure and was calculated by adding one-third of the pulse pressure to the diastolic pressure. The differences between groups were assessed by paired *t*-test or by one-way analysis of variance followed by Student–Newman–Keuls test. Values of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Gastric motor and cardiovascular effects of intravenously administered cannabinoids

The effects of vehicle and Δ^9-THC, administered as a bolus intravenous injection (0.02–2 mg/kg), on gastric motor function are shown in Fig. 1. Δ^9-THC significantly decreased intragastric pressure and pyloric contractile activity at doses of 0.2 and 2 mg/kg. However, significant changes in greater curvature contractile activity were only obtained after Δ^9-THC at the dose of 2 mg/kg.

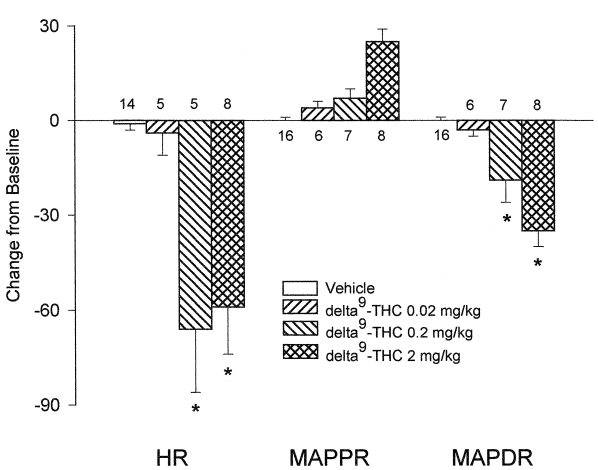

Fig. 2. Prolonged effects of vehicle or Δ^9-THC (0.02–2 mg/kg) administered as a bolus intravenous injection on heart rate (HR) as well as immediate (within 1 min) mean arterial blood pressure (MAPPR) and prolonged depressor responses (MAPDR). Data are means ± S.E. for the number of animals indicated below or above each bar. * Statistically significant when compared with the effect of vehicle.

The changes in cardiovascular function after a bolus intravenous injection of Δ^9-THC at doses of 0.02–2 mg/kg are shown in Fig. 2. Significant decreases in mean arterial blood pressure and heart rate were obtained after Δ^9-THC at doses of 0.2 and 2 mg/kg, but not at a dose of 0.02 mg/kg. The changes in mean arterial blood pressure in response to Δ^9-THC at the highest dose of 2 mg/kg were biphasic and there is a significant (though transient) hypertension at this dose, followed by a prolonged hypotension.

Trace recordings from one experiment in which Δ^9-THC was administered intravenously at a dose of 0.2 mg/kg are shown in Fig. 3A. A peak decrease in intragastric pressure (change from baseline: -1.0 cmH_2O) occurred within 2 min after injection and returned to baseline within 24 min after that (total area of the response: -0.25 cm^2). Corresponding decreases in pyloric (change from baseline: -6.5) and greater curvature contractile activity (change from baseline: -2.0), and in heart rate (change from baseline:

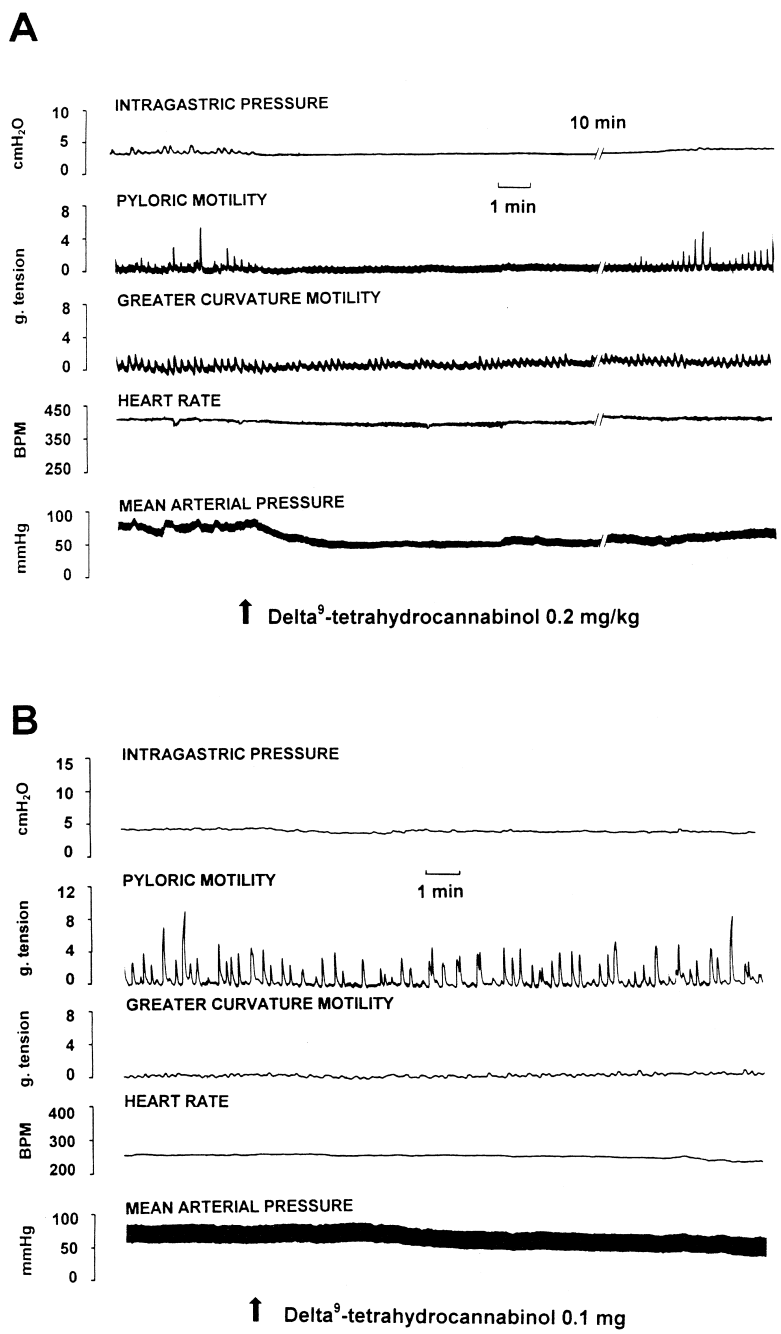

Fig. 3. Representative chart recording from one experiment in which Δ^9-THC was administered intravenously at a dose of 0.2 mg/kg (A) or applied to the dorsal surface of the medulla at a single dose of 0. 1 mg (B).

Table 1
Effects of vehicle and SR141716A (1 mg/kg, i.v.) on intragastric pressure (peak response; PRIGP), pyloric (PCA) and greater curvature GCCA contractile activity as well as on heart rate (HR) and mean arterial blood pressure (MAP) maximum responses. Values are means ± S.E. for the number (*n*) of animals

Treatment	PRIGP (cmH_2O)	PCA (MMI)	GCCA (MMI)	HR (bpm)	MAP (mmHg)
Vehicle	0.0 ± 0.0 (9)	0.4 ± 0.3 (8)	0.2 ± 0.2 (9)	−2 ± 3 (9)	4 ± 2 (9)
SR141716A	−1.5 ± 0.5[a] (9)	−4.4 ± 3.0 (8)	−0.7 ± 1.1 (9)	−30 ± 5[a](9)	41 ± 7[a] (9)

[a]Statistically significant when compared with corresponding mean for vehicle injection.

−30 bpm) with a prolonged depressor blood pressure response (nadir response: −30 mmHg) were also observed in this animal.

3.2. *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide and cannabinoid-evoked gastric motor and/or cardiovascular responses

To determine the involvement of the cannabinoid CB_1 receptor, a putative cannabinoid CB_1 receptor antagonist, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, was administered intravenously at a dose of 1 mg/kg, 15–20 min before Δ^9-THC (2 mg/kg, i.v.). *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide alone evoked transient decreases in intragastric pressure and hypertension that were associated with bradycardia (Table 1). *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide completely blocked the gastric motor and cardiovascular responses to intravenous Δ^9-THC (Table 2). In two additional animals, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (1 mg/kg, i.v.) did not alter the bradycardia and hypotension evoked by cannabidiol (2 mg/kg, i.v., data not shown).

3.3. Vagotomy, spinal cord transection, and ganglionic blockade

The effects of vagotomy, spinal cord transection, and ganglionic blockade on the autonomic responses to Δ^9-THC (0.2–2 mg/kg, i.v.) are shown in Table 3.

Table 2
Effects of SR141716A on peak changes in intragastric pressure (PRIGP), pyloric (PCA) and greater curvature GCCA contractile activity as well as heart rate (HR) and mean arterial blood pressure (MAPPR) and depressor (MAPDR) maximum responses evoked by intravenous administration of Δ^9-THC (2 mg/kg) in five animals. Values are means ± S.E.

Treatment	PRIGP (cmH_2O)	PCA (MMI)	GCCA (MMI)	HR (bpm)	MAPPR (mmHg)	MAPDR (mmHg)
Vehicle	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	−4 ± 2	2 ± 1	2 ± 1
THC before SR141716A	−0.9 ± 0.3[a]	−2.6 ± 1.0[a]	−1.2 ± 0.5	−51 ± 13[a]	27 ± 8[a]	−15 ± 5[a]
THC after SR141716A	0.4 ± 0.4[b]	0.6 ± 0.5[b]	0.6 ± 0.5[b]	0 ± 0[b]	7 ± 3[b]	0 ± 0[b]

[a]Statistically significant when compared with corresponding mean for vehicle administration.
[b]Statistically significant when compared with corresponding mean for THC before SR141716A.

Table 3
Effects of bilateral cervical vagotomy (VAG), spinal cord transection (SCT) and ganglionic blockade with hexamethonium (HEX) on peak intragastric pressure (PRIGP), pyloric (PCA) and greater curvature (GCCA) contractile activity as well as heart rate (HR) and mean arterial blood pressure (MAPPR) and depressor (MAPDR) maximum responses evoked by i.v. administration of Δ^9-THC (0.2–2 mg/kg) for the number (*n*) of animals. Values are means ± S.E.

Treatment	PRIGP (cmH_2O)	PCA (MMI)	GCCA (MMI)	HR (bpm)	MAPPR (mmHg)	MAPDR (mmHg)
Vehicle	0.0 ± 0.0 (7)	0.7 ± 0.4 (7)	−0.1 ± 0.1 (7)	0 ± 0 (7)	0 ± 0 (5)	0 ± 0 (5)
THC	−1.3 ± 0.2[a] (7)	−5.7 ± 1.2[a] (7)	−2.3 ± 0.6[a] (7)	−71 ± 17[a] (7)	32 ± 5[a] (5)	−28 ± 5[a] (5)
VAG + THC	0.2 ± 0.3[b] (7)	0.2 ± 0.7[b] (7)	0.3 ± 0.2[b] (7)	−4 ± 9[b] (7)	35 ± 6[a] (5)	−11 ± 2[a,b] (5)
Vehicle	0.0 ± 0.0 (4)	0.5 ± 0.5 (4)	−0.1 ± 0.1 (4)	0 ± 0 (4)	0 ± 0 (4)	0 ± 0 (4)
THC	−0.8 ± 0.1[a] (4)	−4.4 ± 1.0[a] (4)	−0.9 ± 0.1[a] (4)	−35 ± 12[a] (4)	21 ± 5[a] (4)	−15 ± 2[a] (4)
SCT + THC	−0.8 ± 0.3[a] (4)	−4.0 ± 1.5[a] (4)	−1.3 ± 0.3[a] (4)	−33 ± 8[a] (4)	25 ± 5[a] (4)	−1 ± 1[b] (4)
Vehicle	0.4 ± 0.4 (4)	0.1 ± 0.1 (4)	0.0 ± 0.0 (4)	0 ± 0 (4)	0 ± 0 (4)	0 ± 0 (4)
THC	−0.6 ± 0.1[a] (4)	−5.5 ± 1.7[a] (4)	−0.4 ± 0.4 (4)	−33 ± 10[a] (4)	18 ± 6[a] (4)	−15 ± 5[a] (4)
HEX + THC	0.3 ± 0.1[b] (4)	0.4 ± 0.4[b] (4)	0.0 ± 0.0 (4)	−6 ± 6[b] (4)	10 ± 4 (4)	0 ± 5[b] (4)

[a]Statistically significant when compared with corresponding mean for vehicle administration.
[b]Statistically significant when compared with corresponding mean for THC before VAG, SCT or HEX.

Bilateral vagotomy at midcervical level completely abolished Δ^9-THC-evoked gastric motor inhibition and bradycardia. In contrast, spinal cord transection did not alter the Δ^9-THC-evoked gastric motor inhibition and bradycardia. Vagotomy did not affect the transient initial increase in mean arterial blood pressure, and the prolonged hypotension was still evident, although attenuated when compared with the effect of Δ^9-THC before vagotomy.

Spinal cord transection completely blocked Δ^9-THC-induced hypotension but not the brief initial increase in mean arterial blood pressure.

Ganglionic blockade with hexamethonium bromide completely abolished the decreases in intragastric pressure, pyloric contractile activity, heart rate and mean arterial blood pressure (long-lasting depressor response). However, the immediate pressor response to Δ^9-THC was not affected (Table 3).

In general, vagotomy, spinal cord transection and ganglionic blockade alone did not significantly decrease baseline gastric motor or cardiovascular function (Table 4). Instead, an increase in heart rate after vagotomy and a slight increase in pyloric contractile activity after spinal cord transection were observed.

3.4. Gastric motor and cardiovascular effects of Δ^9-THC in the lower brainstem

The effects of vehicle and Δ^9-THC, applied to the surface of the dorsal medulla at doses of 0.01 and 0.1 mg/rat, on gastric motor and cardiovascular function are shown in Fig. 4. At both doses, Δ^9-THC decreased intragastric pressure (both peak and area of the response). However, a decrease in pyloric contractile activity (Fig. 4A), as well as decreases in heart rate and mean arterial blood pressure (Fig. 4B) achieved statistical significance only after the 0.1 mg/rat dose of Δ^9-THC.

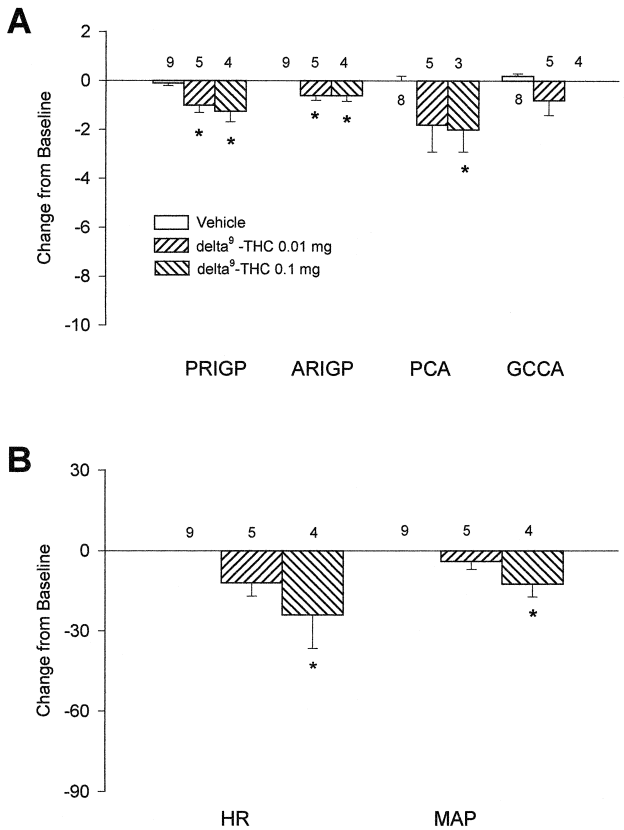

Fig. 4. Acute effects of vehicle or Δ^9-THC applied to the surface of the dorsal medulla of rats at doses of 0.01 and 0.1 mg. (A) Illustrates compiled data showing effects on peak intragastric pressure (PRIGP), pyloric circular muscle (PCA) and greater curvature longitudinal muscle (GCCA) contractile activity. PCA and GCCA values reflect changes which occurred within 2 min after injections. (B) Illustrates changes in heart rate (HR) and mean arterial blood pressure (MAP). Data are means ± S.E. for the number of animals indicated below or above each bar. * Statistically significant when compared with the effect of vehicle.

Trace recordings from a representative experiment in which Δ^9-THC was applied to the surface of the dorsal medulla at a dose of 0. 1 mg are shown in Fig. 3B. In this particular animal, a decrease in intragastric pressure (change from baseline: -1 cmH_2O) appeared immediately after application of Δ^9-THC and persisted for 7 min (total area of the response: -0.32 cm^2). There was also a decrease in pyloric contractile activity (change from baseline: -3.5). The gastric motor changes were accompanied by a decrease in heart rate (change from baseline: -5 bpm) and mean arterial blood pressure (change from baseline: -15 mmHg).

Table 4
Baseline values for intragastric pressure (IGP), pyloric (PCA) and greater curvature (GCCA) contractile activity (calculated as minute motility index) as well as heart rate (HR) and mean arterial blood pressure (MAP) before and 30 min after bilateral vagotomy at midcervical level (seven animals), spinal cord transection (four animals) or ganglionic blockade (four animals). Data are shown as means ± S.E.

Treatment	IGP (cmH_2O)	PCA (MMI)	GCCA (MMI)	HR (bpm)	MAP (mmHg)
Vagotomy					
Before	3.6 ± 0.4	3.4 ± 1.1	1.2 ± 0.4	309 ± 11	69 ± 5
After	3.4 ± 0.3	4.1 ± 1.2	1.4 ± 1.2	391 ± 20[a]	78 ± 7
Spinal cord transection					
Before	2.6 ± 0.2	5.3 ± 1.8	1.3 ± 0.9	313 ± 21	74 ± 6
After	2.6 ± 0.2	6.3 ± 1.9[a]	1.0 ± 0.6	253 ± 18	69 ± 8
Ganglionic blockade					
Before	3.1 ± 0.2	1.4 ± 1.0	0.0 ± 0.0	338 ± 5	90 ± 4
After	3.4 ± 0.3	0.6 ± 0.2	0.1 ± 0.1	350 ± 9	84 ± 6

[a] Statistically significant when compared with corresponding mean before treatment.

4. Discussion

Our study demonstrated that intravenous Δ^9-THC, but not cannabidiol, inhibits gastric motility and decreases intragastric pressure in anesthetized rats. These effects are

abolished by prior administration of the cannabinoid CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, indicating that the autonomic effects of systemically-administered Δ^9-THC are mediated by cannabinoid CB_1 receptors. Bilateral vagotomy and ganglionic blockade abolished the Δ^9-THC-evoked inhibition of gastric motor function. Spinal cord transection does not affect Δ^9-THC-evoked changes in gastric motility but does abolish the Δ^9-THC-evoked depressor response. These data suggest that the effects of Δ^9-THC on gastric motility and intragastric pressure are due to a change in vagal outflow to the stomach, whereas the prolonged decrease in blood pressure is mediated through the sympathetic pathways. Application of Δ^9-THC directly to the dorsal surface of the medulla evokes very slight changes in gastric motor activity and produces short-lasting hypotension and bradycardia. Therefore, a portion of the effects of peripherally-administered Δ^9-THC may be due to a site of action directly in the hindbrain (dorsal vagal complex), although this cannot account for the total magnitude of the observed autonomic effect of intravenously administered Δ^9-THC.

Psychoactive cannabinoids are known to slow gastric emptying and intestinal transit in rodents (Dewey et al., 1972; Chesher et al., 1973; Anderson et al., 1974; Jackson et al., 1976; Shook and Burks, 1989) and in chemotherapy patients (Sridhar et al., 1984). However, to understand the mechanism(s) by which Δ^9-THC may attenuate nausea and vomiting, it is necessary to determine its effects on gastric motility and intragastric pressure. This is because a relationship between nausea and changes in gastric emptying is not always evident (Feinle et al., 1995). On the other hand, gastric volume (Feinle et al., 1995) and gastric motor dysrhythmias (Walsh et al., 1996) are frequently associated with nausea and emesis. In a previous study in rats and mice, Δ^9-THC decreased the frequency of gastric contractions; however, the authors were not able to demonstrate a statistically significant effect of this compound on antral intraluminal pressure in unanesthetized rats (Shook and Burks, 1989). Our study confirms the inhibition of gastric contractility induced by Δ^9-THC and also demonstrated that it decreases intragastric pressure. It is not clear from the work of Shook and Burks (1989) which dose of Δ^9-THC failed to alter intragastric pressure, but from the data presented in the paper there was an initial decrease in antral luminal pressure by about 40%. Thus, our data are not in conflict with the work of Shook and Burks (1989), but slight differences in methodology may account for our ability to demonstrate a significant effect of Δ^9-THC on gastric tone in rats.

It has been previously reported that the non-psychoactive ingredients of marijuana, such as cannabidiol, have little effect on gastric emptying and small intestinal transit in the mice after oral (Chesher et al., 1973; Anderson et al., 1974) or intravenous (Shook and Burks, 1989) administration. We administered one dose of cannabidiol (2 mg/kg, i.v.) to seven animals and overall we found small decreases in gastric motor function which did not achieve statistical significance (data not shown). Therefore, it is unlikely that the non-psychoactive components of marijuana account for the decrease in gastric motor function.

N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, a selective antagonist of the cannabinoid CB_1 receptor subtype with high affinity for the cannabinoid CB_1 receptor (Rinaldi-Carmona et al., 1995), abolished the gastric motor and hypotensive effects of Δ^9-THC. This finding demonstrates that the effect of Δ^9-THC on gastric motor function is mediated through the cannabinoid CB_1 cannabinoid receptor subtype, similar to the case for inhibition of intestinal transit in vivo (Colombo et al., 1998) and electrically-evoked intestinal contractions in vitro (Pertwee et al., 1996; Lopez-Redondo et al., 1997). Our experiments also revealed an immediate and transient inhibitory effect of *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide alone on gastric motor function and heart rate. This agent has been described as an inverse agonist at human recombinant cannabinoid CB_1 (Landsman et al., 1997) and both CB_1 and CB_2 receptors (MacLennan et al., 1998). However, these in vitro systems overexpressed cannabinoid receptors and there is no evidence of such an action in more physiological systems where the receptors are not overexpressed. Since the direction of changes produced by *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide was similar to that of Δ^9-THC, it seems highly unlikely that inverse agonism could account for the effects we observed.

Our observations may raise question about the specificity of *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide at the dose used (1 mg/kg). Nonetheless, when administered intrathecally at doses less and greater than 100 μg/mouse, the compound did not produce either antinociceptive or hyperalgesic effects (Welch et al., 1998). In general, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide has been extensively studied in a variety of systems and appears to be selective for the cannabinoid CB_1 receptor (Felder et al., 1995; Rinaldi-Carmona et al., 1995, 1996b; Showalter et al., 1996; Vivian et al., 1998). The data published by Compton et al. (1996) indicate that the AD_{50} values for the compound are similar to those for peripherally or centrally administered cannabinoids.

The issue of whether a central site of action of Δ^9-THC contributes to its gastrointestinal effects has remained somewhat inconclusive. Δ^9-THC inhibits gastric emptying and small intestinal transit after intracerebroventricular administration in mice (Shook et al., 1986). However, the effective doses of the drug administered centrally are comparable with those used for intravenous route of administration (Shook and Burks, 1989). In the present study,

some of the gastric motor effects of Δ^9-THC were mediated at the level of the dorsal vagal complex. Application of the drug to the dorsal surface of the medulla evoked similar, though shorter-lasting changes in gastric motility and intragastric pressure, compared to those observed after intravenous administration of Δ^9-THC. The most parsimonious explanation for this observation is that Δ^9-THC transiently decreases excitatory vagal motor neuron outflow to the gastric smooth muscle. We can therefore conclude that Δ^9-THC-evoked changes in gastric motor function have a central component, though the extent of involvement of this pathway in the responses to systemically-administered Δ^9-THC seems to be small, at least in terms of the duration of the response. The availability of water-soluble cannabinoid CB_1 receptor agonists and antagonists will enable us to investigate the effects of these compounds in the dorsal vagal complex.

Both ganglionic blockade and vagotomy, but not spinal cord transection, abolished the gastric motor effects of peripherally-administered Δ^9-THC. Taken together, these data indicated that the gastric effects of systemically-administered Δ^9-THC depend on intact vagal circuitry. Thus, in addition to an effect of Δ^9-THC directly on the hindbrain to reduce vagal drive to the gut, it may well act at other sites in vago-vagal circuitry. One likely site of action is vagal afferent endings in the stomach that initiate reflex changes in vagal motor output. Although this hypothesis was not tested in the present investigation, it is based on the known effects of other compounds (e.g., cholecystokinin, Grundy et al., 1995) that mediate changes in gastric motor function via effects on vagal afferent fibers. Furthermore, the effects of Δ^9-THC on pulmonary hemodynamics were previously attributed to reflexogenic mechanisms involving afferent vagi and efferent autonomic pathways (Bright et al., 1975; Jandhyala et al., 1976).

The present study also confirms and extends previous observations on the marked cardiovascular effects of Δ^9-THC. A delayed hypotension (Estrada et al., 1987) and bradycardia (Adams et al., 1976) in response to Δ^9-THC in anesthetized rats is well-known. The decrease in blood pressure is usually preceded by a transient pressor effect which has been attributed to the interaction of Δ^9-THC with cannabinoid CB_2 receptors (Munro et al., 1993), to norepinephrine neurons (Adams et al., 1976; Varga et al., 1995) or to direct vasoconstriction (Siqueira et al., 1979). However, in our study, both ganglionic and cannabinoid CB_1 receptor blockade significantly reduced the hypertension evoked by peripherally-administered Δ^9-THC.

Δ^9-THC-induced bradycardia and hypotension have been attributed to a central site of action resulting in decreased sympathetic tone (Graham and Li, 1973; Cavero et al., 1974; Vollmer et al., 1974). It has been suggested that the inhibitory effect of Δ^9-THC on heart rate results mainly from the activation of the parasympathetic nervous system as well as from the withdrawal of the sympathetic neurogenic tone to the heart (Cavero et al., 1973). Our study suggests that there is a role of the vagus nerve in Δ^9-THC-induced bradycardia, and sympathetic outflow in terms of the hypotension response. Bradycardia in response to Δ^9-THC in the rat was markedly reduced when the cannabinoid was administered after atropine sulfate (Lahiri et al., 1972; Cavero et al., 1973; Graham and Li, 1973) and bilateral vagotomy following atropinization did not produce any further alteration of the response (Cavero et al., 1973). Thus, bradycardia was due to changes in vagal outflow, and the fact that we observed bradycardia in response to application of Δ^9-THC to the dorsal surface of the medulla, suggests that this cannabinoid increases vagal outflow to the heart by a central site of action. Both hypotension and bradycardia evoked by Δ^9-THC were abolished by prior administration of a cannabinoid CB_1 receptor antagonist, similar to a report by Lake et al. (1997).

So far, the functional significance of the cannabinoids for gastrointestinal disorders has focused on their use as antiemetics, especially for management of chemotherapy-related nausea and emesis (Mattes et al., 1993). The present study suggests that Δ^9-THC affects both intragastric pressure and gastric motility, which may contribute to the antiemetic properties of the drug. The sites of action of Δ^9-THC are probably at the level of the dorsal vagal complex, the vagus nerves and within the gastrointestinal tract myenteric plexus. Further investigations, utilizing new selective cannabinoid CB_1 receptor agonists and antagonists, will help elucidate the precise role of each of these sites in the overall spectrum of gastrointestinal and cardiovascular responses to Δ^9-THC.

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